PERSISTENCE OF *Steinernema carpocapsae* AND *S. feltiae* (RHABDITIDA: STEINERNEMATIDAE) IN THE SOIL AGAINST *Agrotis ipsilon* (LEPIDOPTERA: NOCTUIDAE)

Ingrid Schimidt Kaiser[1], Victor Luiz Souza Lima[1], Dirceu Pratissoli[1], Alixelhe Pacheco Damascena[1], Regiane Cristina Oliveira de Freitas Bueno[2]

[1] Universidade Federal do Espírito Santo – UFES, Departamento de Agronomia, Laboratório de Entomologia/NUDEMAFI, Alegre, ES. [2] Universidade Estadual Paulista - UNESP, Faculdade de Ciências Agronômicas Departamento de Proteção Vegetal, Botucatu, SP. E-mail: victor.souzalima@gmail.com

ABSTRACT
The black cutworm *Agrotis ipsilon* (Lepidoptera: Noctuidae) is a polyphagous pest that attacks more than one hundred types of crops. The habit of being buried or underneath the cultural remains may help contact with entomopathogenic nematodes (EPNs), which are natural enemies of insects. The objective of this study was to evaluate the persistence of the pathogenicity of *Steinernema carpocapsae* and *S. feltiae* (Rhabditida: Steinernematidae) applied to the soil against *A. ipsilon* larvae. The EPNs were applied to vases containing soil and larvae of *A. ipsilon* were introduced into the vases in five periods (0, 2, 4, 6, and 8 days after treatment). The two species of EPNs caused pathogenicity in all evaluated periods. The mortality rate was over 92% in all treatments and periods evaluated. Based on the results, it is possible to indicate applications NEPs every eight days for larvae population reduction of *A. ipsilon*.
Keywords: biological control; black cutworm; entomopathogenic nematodes; infective juvenile.

PERSISTÊNCIA DE *Steinernema carpocapsae* E *S. feltiae* (RHABDITIDA: STEINERNEMATIDAE) NO SOLO SOBRE *Agrotis ipsilon* (LEPIDOPTERA: NOCTUIDAE)

RESUMO
A lagarta rosca *Agrotis ipsilon* (Lepidoptera: Noctuidae) é uma praga polífaga que ataca mais de uma centena de tipos de cultivos. O hábito de ficar enterrada ou abaixo dos restos culturais pode ajudar o contato com nematoides entomopatogênicos (NEP), que são inimigos naturais de insetos. O objetivo deste estudo foi avaliar a persistência da patogenicidade de *Steinernema carpocapsae* e *S. feltiae* (Rhabditida: Steinernematidae), aplicados no solo contra lagartas de *A. ipsilon*. Os NEPs foram aplicados em vasos contendo solo e lagartas de *A. ipsilon* foram introduzidas nos vasos em cinco períodos (0, 2, 4, 6, e 8 dias após o tratamento). As duas espécies de NEPs causaram patogenicidade em todos os períodos avaliados. A taxa de mortalidade foi superior a 92% em todos os tratamentos e períodos avaliados. Com base nos resultados, é possível indicar aplicações de NEPs a cada oito dias para redução populacional de lagartas de *A. ipsilon.*
Palavras-chave: controle biológico; juvenis infectivos; lagarta-rosca; nematoides entomopatogênicos.

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) is a polyphagous pest that attacks more than one hundred types of crops (BUSCHING; TURPIN, 1976), caterpillars cut as the seedlings in early development, causing reduction in the stand. The larval stage has nocturnal habit and remain buried in the soil, below the crop remains or near the host during the day (FERNANDES et al., 2013; LINK; COSTA, 1984). The habit of *A. ipsilon* to remain in soil can make control difficult, however, it facilitates contact with soil microorganisms such as entomopathogenic nematodes (EPNs) (Rhabditida: Steinernematidae and Heterorhabditidae), which are its natural enemies (SEAL et al., 2010).

EPNs are insect parasites and establish a symbiotic relationship with bacteria generally of the genres *Xenorhabdus* and *Photorhabdus* that cause insect pathogenicity (ALMENARA et al.,

2011). Upon penetrating inside the insect hemocele, the EPN releases bacterial cells into the hemolymph and produce toxins capable of killing the host within 48 h (BATHON, 1996; VOSS et al., 2009). EPN feeds occur through the nutrients released in the digestion of host tissues and the bacteria themselves that proliferate in the host's cadaver (BATHON, 1996). When there are no more nutrients, the EPN looks for new hosts (ALMENARA et al., 2011). These biological control agents stand out for the potential of persistence in the soil for a certain period of time, which ensures control over the pests days after its application to the soil (EBSSA; KOPPENHÖFER, 2011). The persistence of infective juvenile (IJ), the stage of free life of the nematode, is due to the presence of two overlying cuticles, tolerant to different pesticides and fertilizers (GREWAL et al., 2001; KAYA; GAUGLER, 1993).

EPNs have a lot of potential for biological pest control programs, however studies showing the persistence of EPNs in soil are scarce. Thus, the objective of the present work was to determine the persistence of the pathogenicity of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) and *S. feltiae* applied to the soil against *A. ipsilon*.

MATERIAL AND METHODS

For the experiments, a *A. ipsilon* rearing was established at the Laboratório de Entomologia (NUDEMAFI) of the Universidade Federal do Espírito Santo (UFES/CCAE). The insects were provided by the Núcleo de Estudos em Manejo Integrado de Pragas Agrícolas (AGRIMIP) of the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP/Botucatu). The insect multiplication was carried out in an air-conditioned room, with a temperature of 25 ± 1 ºC, RH 70 ± 10% and photophase 14 h. Adult moths were placed in PVC cages (25 cm diameter x 25 cm height). The inside of the cages was paper coated, the top end was closed with paper towel and the bottom end was coated with styrofoam sheet (25 x 25 x 3 cm thickness). The moths were fed a solution of honey (10%). The paper covering the cages and containing the oviposition's moth was packed in plastic pots (1 L). After hatching, the caterpillars were transferred to plastic pots (50 mL). The caterpillars were fed an artificial diet of Greene et al. (1976). Nine-day-old caterpillars were individualized in plastic pots (3 cm diameter) and fed with diet until the pupal stage. The pupae were transferred to acrylic cages (50 x 50 x 50 cm) until adult emergence.

EPNs *S. feltiae* and *S. carpocapsae* were assigned by Koppert Biological Systems, located in Piracicaba, São Paulo, Brazil. The soil persistence bioassay was performed with three treatments: *S. feltiae*, *S. carpocapsae* and control. The bioassay was carried out in plastic vases (2.5 L) under greenhouse conditions. The substrate containing soil, bovine manure and sand in the proportion 3: 1: 1.5 was placed in the vases and moistened with distilled water during the bioassay. The EPN solution was adjusted to concentration of 150 infective juveniles.cm^{-2}, as proposed by Giannasi (2014). The EPN solution was pipetted into the volume of 5 mL per vase. In the control was applied 5 mL distilled water. Each repetition consisted of a vase. Ten vases were used in each treatment. To evaluate the persistence period of EPNs in the soil, 3rd instar larvae of *A. ipsilon* were introduced into the soil at 0, 2, 4, 6, and 8 days after treatment (DAT). The larvae were individually placed in punctured Eppendorf tubes (1.5 mL) containing artificial diet. Five tubes were placed in each vase. The tubes were removed from the soil after 48 h and transferred to plastic boxes (11 x 11 x 3.5 cm) containing artificial diet. To verify the susceptibility of the larvae to the EPNs, the number of dead larvae was recorded for five days. The EPN persistence bioassay and the effect on *A. ipsilon* mortality was performed under temperature conditions of 21.5 ± 4.7 °C.

The bioassay was conducted in a completely randomized design, in a plot scheme subdivided in time with three treatments and five periods of persistence. Mortality data were submitted to analysis of variance and means were compared by the Tukey test ($p < 0.05$). For the analyzes, the package expdes.pt in R software (R DEVELOPMENT CORE TEAM, 2017) was used. (R DEVELOPMENT CORE TEAM, 2017; FERREIRA et al., 2018).

RESULTS AND DISCUSSION

The two species of EPNs caused pathogenicity in all evaluated periods. Analysis of variance showed no significant effect between treatment and persistence period for larval mortality ($p > 0.05$; Tab. 1). However, among treatments there was a significant difference ($p < 0.01$; Tab. 1).

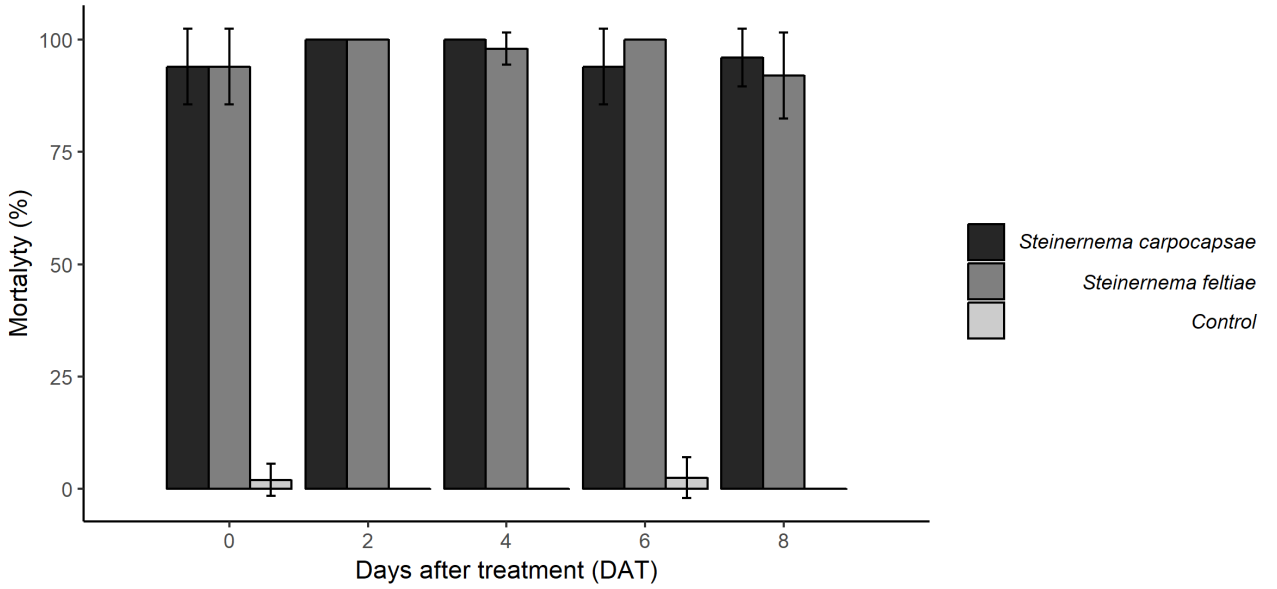
Table 1. Summary of variance analysis for mortality of *Agrotis ipsilon* larvae in different periods of persistence in soil after treatment with entomopathogenic nematodes.

Source of Variation	DF[1]	MS[2]	*p*[3]
Treatment	2	153280	0.00
Error a	27	33	
Persistence period	4	91	0.07614
Treatment * Persistence	8	74	0.08811
Error b	108	42	
Total	149		
CV[4] parcel: 8.91% CV sub parcel: 9.96%			

[1] DF: Degree of freedom; [2] MS: Mean square; [3] *p*: Significance level ($p < 0.01$); [4] CV: Coefficient of Variation.

In the present study, we observed that EPNs were persistent in the soil for eight days after treatment (Figure 1). In addition, the two EPN species caused mortality above 92% in *A. ipsilon* larvae at all persistence periods (Figure 1). This result demonstrates that EPNs are pathogenic *to A. ipsilon* and may persist in soil with pathogenicity potential for eight days after treatment. Among the factors that influence the persistence of nematodes in the soil are temperature, humidity, soil texture, ultraviolet radiation and presence of host (SHAPIRO-ILAN et al., 2006). The ideal temperature range for soil EPN survival is between 15 and 28 °C (AKHURST; SMITH, 2002; GREWAL et al., 1994), interval which covers the temperature of 21.5 ± 4.7 °C recorded in this study and which may have had a positive influence on the persistence period. Soil moisture may also have influenced the pathogenicity of nematodes, since the soil was kept moist during the bioassay, providing conditions for the nematodes to move and remain viable (GRANT; VILLANI, 2003; ROHDE et al., 2010).

Figure 1. Percentage of mortality of *Agrotis ipsilon* larvae by entomopathogenic nematodes.



The persistence and efficacy of biological agents in the environment can ensure the success of their use in biological control programs (ALVES, 1998). The high mortality rate caused by the EPNs (> 92%) on *A. ipsilon* demonstrates the potential of *S. carpocapse* and *S. feltiae*, also found in other

studies that recorded mortalities higher than 70% in *A. ipsilon* larvae (EBSSA; KOPPENHÖFER, 2011, 2012; GIANNASI, 2014; SEAL et al., 2010).

With the results obtained in this study it is possible to affirm that the two species studied, *S. carpocapse* and *S. feltiae*, present pathogenicity to *A. ipsilon*, for eight days after application of the treatment. Therefore, it is possible to indicate a range of application of EPNs every eight days for the biological control of *A. ipsilon*. However, further studies should be carried out to verify the response of EPNs to different types of soil and crops.

ACKNOWLEDGEMENTS

The authors would like to thank the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), to Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES), and to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

REFERENCES

AKHURST, R.; SMITH, K. **Regulation and safety. In: Entomopathogenic nematology**. Wallingford: CABI, 2002. p. 311–332. https://doi.org/10.1079/9780851995670.0311

ALMENARA, D.P.; NEVES, M.R. DE C.; KAMITANI, F.L.; WINTER, C.E. Nematoides entomopatogênicos: as duas faces de uma simbiose. **Revista da Biologia**, v. 6b, n. 2, p. 1–6, 23 jun. 2011.

ALVES, S.B. Controle microbiano de insetos. 2. ed. Piracicaba: FEALQ, 1998.

BATHON, H. Impact of Entomopathogenic Nematodes on Non-target Hosts. Biocontrol Science and Technology, v. 6, n. 3, p. 421–434, 28 set. 1996. https://doi.org/10.1080/09583159631398

BUSCHING, M.K.; TURPIN, F.T. Oviposition Preferences of Black Cutworm Moths Among Various Crop Plants, Weeds, and Plant Debris. **Journal of Economic Entomology**, v. 69, n. 5, p. 587–590, 1 out. 1976. https://doi.org/10.1093/jee/69.5.587

EBSSA, L.; KOPPENHÖFER, A.M. Efficacy and persistence of entomopathogenic nematodes for black cutworm control in turfgrass. **Biocontrol Science and Technology**, v. 21, n. 7, p. 779–796, 6 jul. 2011. https://doi.org/10.1080/09583157.2011.584610

EBSSA, L.; KOPPENHÖFER, A.M. Entomopathogenic nematodes for the management of Agrotis ipsilon: effect of instar, nematode species and nematode production method. **Pest Management Science**, v. 68, n. 6, p. 947–957, jun. 2012. https://doi.org/10.1002/ps.3259

FERNANDES, F.L.; DINIZ, J.F.S.; SILVA, P.R.; MOSCA, E. Damage of *Agrotis ipsilon* (Lepidoptera: Noctuidae) on Coffea arabica in Brazil. **Revista Colombiana de Entomología**, v. 39, n. 1, p. 49–50, 2013.

FERREIRA, E.B.; CAVALCANTI, P.P; NOGUEIRA, D. A. ExpDes.pt: **Pacote Experimental Designs (Portuguese)**. R package version 1.2.0. https://CRAN.R-project.org/package=ExpDes.pt. 2018

GIANNASI, A.O. **Aspectos biológicos de *Agrotis ipsilon* (Lepidoptera: Noctuidae) (Hufnagel, 1767) e testes com nematoides entomopatogênicos (Rhabditida: Steinernematidae e Heterorhabditidae) visando o seu controle**. Universidade Estadual do Norte do Paraná, 2014. 67 f.

GRANT, J.A.; VILLANI, M.G. Effects of Soil Rehydration on the Virulence of Entomopathogenic Nematodes. **Environmental Entomology**, v. 32, n. 5, p. 983–991, 1 out. 2003. https://doi.org/10.1603/0046-225X-32.5.983

GREENE, G.L.; LEPPLA, N.C.; DICKERSON, W.A. Velvetbean caterpillar: a rearing procedure and artificial medium. **Journal of Economic Entomology**, v. 69, n. 4, p. 487–488, 1976. https://doi.org/10.1093/jee/69.4.487

GREWAL, P.S.; NARDO, E.A.B. DE; AGUILLERA, M.M. Entomopathogenic Nematodes: Potential For Exploration and Use in South America. **Neotropical Entomology**, v. 30, n. 2, p. 191–205, 1 jun. 2001. https://doi.org/10.1016/0306-4565(94)90047-7

GREWAL, P.S.; SELVAN, S.; GAUGLER, R. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, breeding and

reproduction. **Journal of thermal biology**, v. 19, n. 4, p. 245–253, 1994.

KAYA, H.K.; GAUGLER, R. Entomopathogenic Nematodes. **Annual Review of Entomology**, v. 38, n.1, p.181–206, 28 jan. 1993. https://doi.org/10.1146/annurev.en.38.010193.001145

LINK, D.; COSTA, E.C. Comportamento larval da lagarta-rosca, *Agrotis ipsilon* (Hufnagel, 1767). **Revista do Centro de Ciências Rurais**, v. 14, n. 3–4, p. 191–199, 1984.

R DEVELOPMENT CORE TEAM. **R: A language and environment for statistical computing**. Vienna: AustriaR Foundation for Statistical Computing, , 2017.

ROHDE, C.; MOINO, A.; SILVA, M.A.T. DA; CARVALHO, F.D.; FERREIRA, C.S. Influence of soil temperature and moisture on the infectivity of entomopathogenic nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) against larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). **Neotropical entomology**, v.39, n.4, p.608–11, 2010. https://doi.org/10.1590/S1519-566X2010000400022

SEAL, D.R.; JHA, V.K.; LIU, T.X. Potential of various strains of Entomopathogenic Nematodes in combination with Insecticides for suppression of Black Cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae). **Annals of Plant Protection Sciences**, v. 18, n. 2, p. 293–300, 2010.

SHAPIRO-ILAN, D.I.; GOUGE, D.H.; PIGGOTT, S.J.; FIFE, J.P. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. **Biological Control**, v. 38, n. 1, p. 124–133, jul. 2006. https://doi.org/10.1016/j.biocontrol.2005.09.005

VOSS, M.; ANDALÓ, V.; NEGRISOLI JÚNIOR, A.S.; BARBOSA-NEGRISOLI, C.R. **Manual de técnicas laboratoriais para obtenção, manutenção e caracterização de nematoides entomopatogênicos**. Documentos ed. Passo Fundo: Embrapa Trigo, 2009. 44p.

Recebido para publicação em 20/06/2018
Revisado em 14/01/2019
Aceito em 14/01/2019